

## UV Study of the Protonation of Indole-2-carboxylic Acid, 3-Methylindole, 3-Acetylindole and D-Tryptophan in Perchloric Acid Solutions

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Protonation of indole-2-carboxylic acid, 3-methylindole, 3-acetylindole and D-tryptophan in perchloric acid media was studied by UV spectroscopy methods in the 400–190 nm region. Absorbance values were measured at four selected wavelengths and the molar absorptivity was calculated. From these values, the  $pK_a$ 's were determined using Hammett's equation. Also, the dissociation constants and solvent parameter  $m^*$  were obtained by the Excess Acidity Method. The position of additional protons in protonated compounds was discussed.

**Key words:** UV spectra, protonation, indole-2-carboxylic acid, 3-methylindole, skatole, 3-acetylindole, D-tryptophan, dissociation constants, Hammett, acidity functions, excess acidity

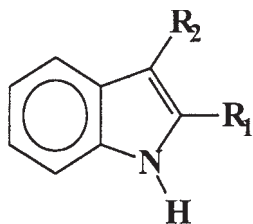
### INTRODUCTION

Indole derivatives have been the subject of extensive research, since many naturally occurring compounds contain the indole ring, for example, the essential amino acid tryptophan, a constituent of almost all proteins, and the class of natural products known as indole alkaloids.<sup>1</sup> We have studied acid-base properties of indole-3-acetic acid (IAA), indole-3-propionic acid (IPA) and indole-3-butyric acid (IBA)<sup>2</sup> known as plant hormones.<sup>3–6</sup> The aim of this study is to determine the dissociation constants,  $pK_a$ , of in-

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dole derivatives in perchloric acid media using the Hammett Acidity Function Method (HAFM)<sup>7-9</sup> and Excess Acidity Method (EAM).<sup>8-11</sup> Similar stud-



$R_1 = \text{H}; R_2 = \text{CH}_3$  (3-MI)

$R_1 = \text{H}; R_2 = \text{CH}_2\text{CHNH}_3^+\text{COO}^-$  (D-Trp)

$R_1 = \text{H}; R_2 = \text{COCH}_3$  (3-AI)

$R_1 = \text{COOH}; R_2 = \text{H}$  (I-2CA)

ies of indole and its methyl derivatives, in strongly acidic media, were performed by Hinman *et. al.*,<sup>12-13</sup> Berti *et. al.*,  $\text{p}K_a$  of skatole -3,35 (Ref. 14) and Cohen and Cohen.<sup>15</sup> Podkowinska *et. al.*<sup>16</sup> and Nowotarska *et. al.*<sup>17-19</sup> studied proton-acceptor properties of 3-substituted indole derivatives in  $\text{HClO}_4$  and  $\text{H}_2\text{SO}_4$  solutions or oleum ( $\text{H}_2\text{SO}_4 \times \text{SO}_3$ ). Neither of the four compounds that are the subject of this paper was among the compounds studied by them. A UV analysis of indole, IAA, IPA and IBA in  $\text{HClO}_4$  in sulphuric acid media protonation was reported separately.<sup>20</sup>

## EXPERIMENTAL

### Materials

Indole-2-carboxylic acid (I-2CA), 3-methylindole (3-MI), 3-acetylindole (3-AI, obtained under the name 3-indolyl-methyl-ketone) and D-tryptophan (D-Trp) were MERCK products and were used without further purification. Physical properties of these compounds (UV absorption spectra and infrared spectra) were in good agreement with the literature values. Purity of the 3-methylindole was further checked by gas-chromatography, on a PERKIN ELMER GC-F17 instrument equipped with hydrogen flame ionization detector.

Reagent grade perchloric acid (70% by weight, product of ALKALOID-Skopje) was used. The concentration of perchloric acid was determined by titration against sodium hydroxide.

Solutions of concentration  $0.001 \text{ mol dm}^{-3}$  were prepared from I-2CA, 3-MI, 3-AI and D-Trp in mixtures of water and ethanol (10% *v/v*). Other more diluted solutions were prepared from these solutions by keeping the concentration of I-2CA, 3-MI, 3-AI and D-Trp constant and varying the concentration of perchloric acid (between 0.1 and  $11.0 \text{ mol dm}^{-3}$ ).

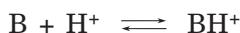
The degree of dilution of the solutions was chosen in such a way that the absorbance had a value between 0.1 and 1.0 at the studied wavelength.

### UV Absorption Spectra

The UV spectra of each solution were recorded between 400 nm and 190 nm on a Hewlett-Packard 8452A Diode Array Spectrophotometer (the scan time per full spectrum is about 0.1 s). The absorbances were measured in 1 cm long quartz cells at four analytical wavelength. Perchloric acid, of the same concentration as in the investigated solutions, was used as a blank. The measurements were taken 1–1.5 min after preparing the solution at  $27 \pm 0.2$  °C in a thermostated cell. Use of extrapolations was avoided.

### The Hammett Acidity Function Method (HAFM)<sup>7–9</sup>

For very weak uncharged bases which undergo monoprotection in strongly acidic media,



BH<sup>+</sup> is called conjugated acid; acidity constants can be expressed in terms of the acidity function  $H_0$

$$pK_a = H_0 + \log I \quad (1)$$

where  $I$  is the ionization ratio  $c(BH^+)/c(B)$  and  $H_0 = -\log(a_{H^+}f_{H^+}/f_{BH^+})$ ,  $c$  is the formal molar concentration,  $a$  is activity, and  $a_{H^+} = c_{H^+}f_{H^+}$ , and  $f$  is the activity coefficient.

As data have accumulated, it has become evident that thus defined acidity functions are valid only for very weak bases closely related in structure.

Nowdays,  $H_0$  is restricted to acidity functions obtained using nitro-substituted primary aromatic amines<sup>21</sup> and new functions have been developed, e.g.  $H_0^m$  for tertiary aromatic amines,<sup>22</sup>  $H_i$  for indoles,<sup>13</sup>  $H_a$  for amides<sup>23</sup> .... The need to take account of solvation has been recognized. Correction for medium effects by characteristic vector analysis has been proposed by Edward and Wong.<sup>24</sup>

### The Excess Acidity Method (EAM)<sup>8–11</sup>

The difficulty that the ratio  $f_B/f_{BH^+}$  varies significantly with the functional groups being protonated and, to a lesser degree, with changing substituents on a given functional group, has been overcome by introducing a two parameter acidity scale:

$$\log I - \log c_{H^+} = m^*X + pK_a \quad (2)$$

The quantities  $m^*$  and  $pK_a$  are unknowns, characteristic of each base, and  $X$  is an unknown characteristic of the solvent but the same for all bases. An iterative analysis of data for a number of bases over the range of pure water to pure strong mineral acid, yields values for the unknowns. Calculations were performed using the program.<sup>10,25</sup>

## RESULTS AND DISCUSSION

Table I presents the four wavelengths and molar absorptivities of the examined compounds, used for dissociation calculations in perchloric acid solutions.

TABLE I

Wavelengths (with molar absorptivity) in perchloric acid solutions of 3-methylindole, 3-acetylindole, D-tryptophan and indole-2-carboxylic acid used for dissociation calculations

cmpds	$c(\text{HClO}_4)$ $\text{mol dm}^{-3}$		$\lambda$ and nm	$\log \varepsilon$ $\text{mol}^{-1} \text{dm}^3 \text{cm}^{-1}$	
3-MI	1.0	200 (4.19)	210 (4.18)	222 (4.46)	232 (4.02)
	9.5	(4.08)	(3.87)	(3.67)	(3.71)
D-Trp	1.0	208 (4.29)	214 (4.41)	220 (4.45)	226 (4.20)
	10.5	(3.89)	(3.59)	(3.48)	(3.52)
3-AI	1.0	280 (3.75)	300 (4.00)	320 (3.62)	340 (2.65)
	9.5	(3.14)	(3.41)	(3.93)	(4.14)
I-2CA	1.0	300 (4.19)	310 (3.84)	320 (3.66)	330 (3.26)
	9.5	(4.03)	(4.00)	(3.95)	(3.90)

TABLE II

Absorbance (UV spectrum) *vs.* concentration data for 3-methylindole

$c(\text{HClO}_4)$ $\text{mol dm}^{-3}$	A (200 nm)	A (210 nm)	A (222 nm)	A (232 nm)
1.0	0.6546	0.6402	1.1640	0.4228
1.5	0.7571	0.7316	1.2150	0.4649
2.0	0.6655	0.6610	1.1500	0.4198
2.5	0.6738	0.6697	1.1620	0.4338
3.0	0.6534	0.6470	1.1200	0.4278
3.5	0.6399	0.6379	1.1000	0.4189
4.0	0.5943	0.6050	1.0560	0.4066
4.5	0.6716	0.6371	1.0360	0.4158
5.0	0.6160	0.5720	0.9262	0.3686
5.5	0.5968	0.4974	0.6775	0.3291
6.0	0.5477	0.4092	0.4262	0.2753
7.0	0.4990	0.3234	0.2397	0.2124
7.5	0.4558	0.2944	0.1772	0.1877
8.0	0.4506	0.2779	0.1492	0.1823
8.5	0.3982	0.2505	0.1162	0.1613
9.0	0.3347	0.1873	0.0430	0.0788

Table II presents absorbance *vs.* concentration data for 3-MI in perchloric acid solutions, measured at 200, 210, 222 and 232 nm.

*UV Spectra of 3-Methylindole and D-Tryptophan in Perchloric Acid Solutions*

The UV spectra of 3-MI and D-Trp in water are very similar. They exhibit four bands which result from  $\pi \rightarrow \pi^*$  transitions in the indolic ring and they are given in Table III.

The positions, intensities and profiles of the spectra are in agreement with those previously reported for 3-MI and tryptophan derivatives<sup>13,26,27</sup>

TABLE III

Electronic transitions in water solutions. Concentrations of the compounds in water were  $4.0 \cdot 10^{-5}$  mol dm<sup>-3</sup>

media	water		
compounds	$\frac{\lambda}{\text{nm}}$	$\frac{\log \varepsilon}{\text{mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}}$	transitions
3-MI	198	4.25	<sup>1</sup> B <sub>a</sub>
	222	4.45	<sup>1</sup> B <sub>b</sub>
	280	3.70	<sup>1</sup> L <sub>a</sub>
	288(sh)	3.67	<sup>1</sup> L <sub>b</sub>
D-Trp	200	4.25	<sup>1</sup> B <sub>a</sub>
	218	4.45	<sup>1</sup> B <sub>b</sub>
	280	3.70	<sup>1</sup> L <sub>a</sub>
	288(sh)	3.66	<sup>1</sup> L <sub>b</sub>
3-AI	208	4.38	<sup>1</sup> B <sub>a</sub>
	242	4.02	<sup>1</sup> B <sub>b</sub>
	260	3.93	<sup>1</sup> L <sub>a</sub>
	300	4.03	<sup>1</sup> L <sub>b</sub>
I-2CA	202	4.23	<sup>1</sup> B <sub>a</sub>
	218	4.26	<sup>1</sup> B <sub>b</sub>
	292	4.22	<sup>1</sup> L <sub>a</sub> <sup>1</sup> L <sub>b</sub>
*I-3Ald	208	4.37	<sup>1</sup> B <sub>a</sub>
	244	4.06	<sup>1</sup> B <sub>b</sub>
	262	4.03	<sup>1</sup> L <sub>a</sub>
	300	4.07	<sup>1</sup> L <sub>b</sub>

\* indole-3-carboxaldehyde

and closely resemble those of indole, IAA, IPA and IBA recorded under the same conditions.<sup>2</sup> The absorption maxima at 216 and 270 nm show bathochromic shifts to 222, 280 nm and 218, 278 nm for 3-MI and D-Trp, respectively. This effect might be readily explained by hyperconjugation electron release from the methyl group. The  $^1L_b$  band in this spectral region is actually considerably overlapped with the  $^1L_a$  band. The increase of the  $\text{HClO}_4$  concentration above  $4.5 \text{ mol dm}^{-3}$  has a significant influence on the spectral appearance (Table II and Figure 1). The changes observed for D-Trp are similar to those observed in the spectrum of 3-MI, indole, IAA, IPA and IBA in perchloric acid.<sup>2</sup> In Table IV, characteristic bands of the UV spectra of 3-MIH<sup>+</sup> and D-TrpH<sup>+</sup> are given. For comparison, the literature data for the experimentally obtained transitions in the UV spectra of 3-MIH<sup>+</sup><sup>13,14</sup> and IAAH<sup>+</sup><sup>28</sup> in sulfuric acid are given as well.

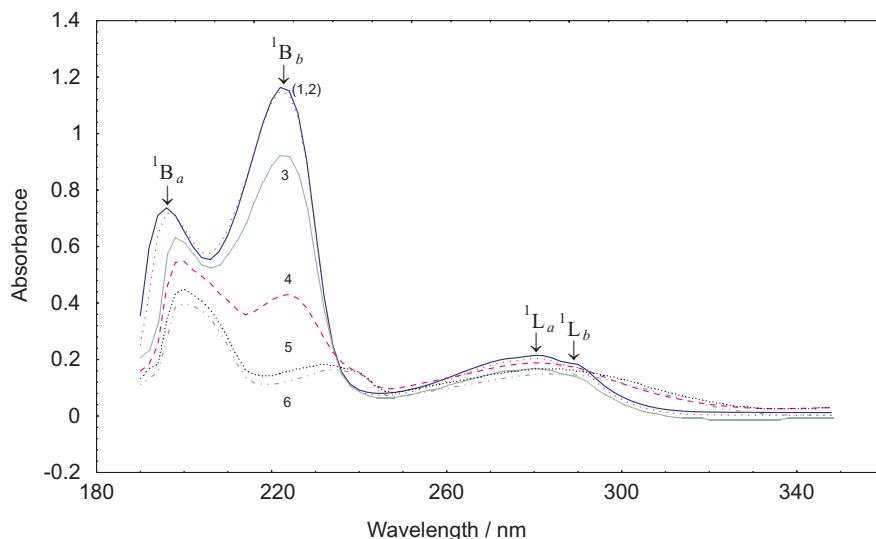


Figure 1. Ultraviolet absorption spectra of 3-methylindole ( $4.0 \cdot 10^{-5} \text{ mol dm}^{-3}$ ) as a function of perchloric acid concentration. Solvent (top to bottom, at  $\lambda = 222 \text{ nm}$ ) perchloric acid: (1)–1.0; (2)–2.0; (3)–5.0; (4)–6.0; (5)– 8.0 and (6)–8.5  $\text{mol dm}^{-3}$ .

Bands at 288 and 290 nm in the spectra of MIH<sup>+</sup> and D-TrpH<sup>+</sup> ions, respectively, are due to the intramolecular charge-transfer transition from the highest occupied orbital ( $H_B$ ) of one part of the ion (phenylene ring), which acts as an electron-donor, to the lowest unoccupied vacant orbital ( $V_S$ ) of the other part (iminium group), which serves as an electron acceptor. This transition was denoted  $H_B \rightarrow V_S$  by Nagakura and Tanaka<sup>29</sup> or HOMO (Highest

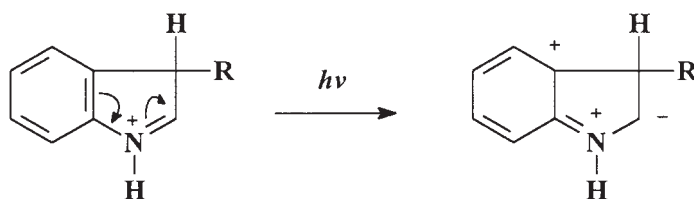
TABLE IV  
Experimental transitions in the UV spectra of MIH<sup>+</sup> and D-TrpH<sup>+</sup>

ions	3-MIH <sup>+</sup>	D-TrpH <sup>+</sup>	3-MIH <sup>+</sup>	3-MIH <sup>+</sup>	IAH <sub>2</sub> <sup>+</sup>	
transitions	$\lambda$ / nm					
<sup>1</sup> L <sub>b</sub>	288	290	290 <sup>1</sup>	285–7 <sup>2</sup>	295.5 <sup>3</sup>	291.3, 271.5 <sup>3</sup>
<sup>1</sup> L <sub>a</sub>	–	–	–	–	255.0	259.2
	240(sh)	240(sh)	241	240	240	–
<sup>1</sup> B <sub>b</sub>	234	234	236	236	231	213.2
<sup>1</sup> B <sub>a</sub>	200	202	–	–	–	–

<sup>1</sup>Ref. 14, <sup>2</sup>Ref. 13, <sup>3</sup>Ref. 28.

Occupied Molecular Orbital) → LUMO (Lowest Unoccupied Molecular Orbital).

On the other hand, the bands in the short wavelength range (in both compounds they appear at ~200, 234 and 240 nm) are probably due to transitions within the phenylene ring. The UV absorption corresponding to the intramolecular charge-transfer transition may be represented by the following scheme:



Scheme 1

The set of spectra of 3-MI in perchloric acid show a few ill-defined isobestic points (Figure 1). In the region of acidities, where 3-MI is expected to be present as ionized base, there is a small bathochromic shift. The effect may be due to the non-constant value of the molar absorptivity for the base and its conjugated acid.<sup>24</sup> The non-constant value of molar absorptivity results from the changes in solvent concentration.

In the case of the UV spectra of indoles in perchloric acid solutions, several factors governing the appearance of isobestic points are of particular importance. Apart from experimental errors, protonation, hydrolysis, solvent shifts, etc. are all possible and are independent of each other. Application of characteristic vector analysis (CVA)<sup>24</sup> to separate these factors is

therefore desirable. Its application shows that the first characteristic vector accounts for 97.98% of the variability. The second characteristic vector accounts for 1.35% of the variability. The curves reconstituted from the mean and the first characteristic vector (Figure 2), now form a few well-defined isobestic points (at 236, 256 and 290 nm). The presence of isobestic points indicates that there is no significant build-up of intermediates.

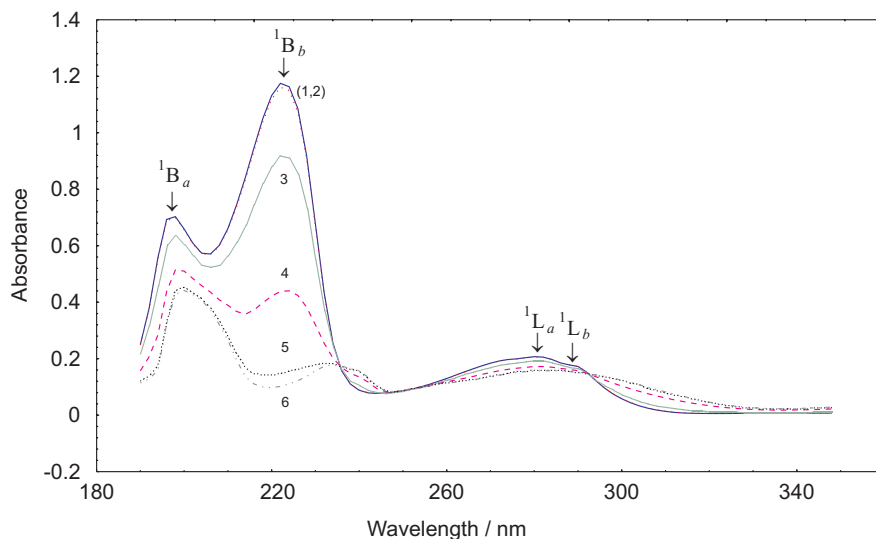
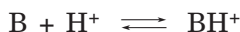


Figure 2. Ultraviolet absorption spectra of 3-methylindole ( $4.0 \cdot 10^{-5} \text{ mol dm}^{-3}$ ) as a function of perchloric acid concentration. Solvent (top to bottom, at  $\lambda = 222 \text{ nm}$ ) perchloric acid: (1)–1.0; (2)–2.0; (3)–5.0; (4)–6.0; (5)–8.0 and (6)–8.5  $\text{mol dm}^{-3}$ . Spectra reconstituted from the mean curve and first characteristic vector.

These results give strong evidence that the first characteristic vector results from the effect of protonation, so it is safe to deal with a single equilibrium.



More extensive results from the application of CVA to a number of indoles will be presented in a subsequent paper.<sup>30</sup>

Plots of absorbance (measured at  $\lambda = 222 \text{ nm}$ ) *vs.* the  $H_i$  acidity function for 3-MI in  $\text{HClO}_4$  give a nonlinear curve (Figure 3), indicating a decrease in the concentration of the unprotonated form (in the range of  $-3.0$  to  $-5.5 H_i$ ). The curve shown in Figure 3 is similar also for D-Trp. In this case, changes in the concentration of the unprotonated form are observed in the range of  $-5.0$  to  $-7.5 H_i$ .



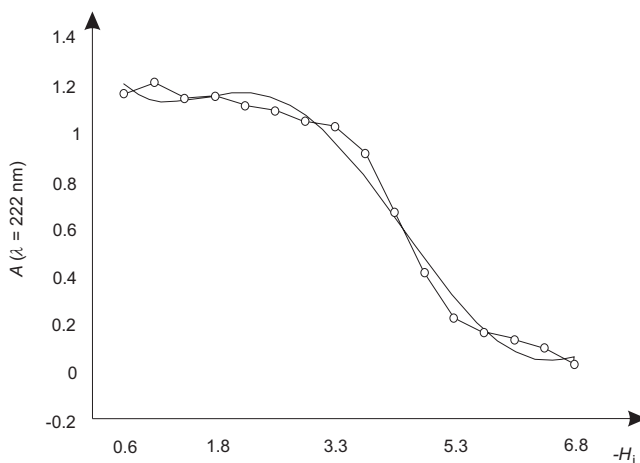


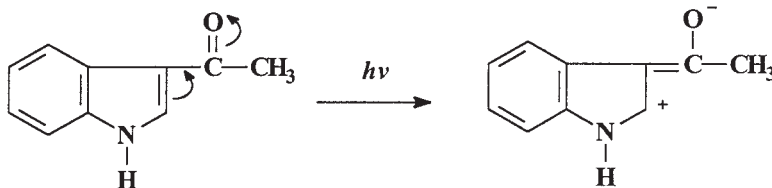
Figure 3. Effect of the  $H_0$  acidity function on the absorbance at 222 nm for 3-methylindole in perchloric acid.

*UV Spectra of 3-Acetylindole (3-AI) and Indole-3-Carboxaldehyde (I-3Ald) in Water and Perchloric Acid Solutions*

As expected, the UV spectra of 3-AI and I-3Ald in water solution have rather a similar appearance (Table III). In both compounds, the carbonyl group is the vicinal one to the indolic ring. The experimentally detected bands at 300 nm ( $^1L_b$ ) and 300 nm ( $^1L_b$ ), for 3-AI and I-3Ald, respectively, are most probably due to the intramolecular charge-transfer transition from the HOMO ( $H_x$ ) of the indolic ring to the LUMO ( $V_A$ ) of the carbonyl group. The corresponding UV absorption may be represented by Scheme 2.

The band at about 260 nm ( $^1L_a$ ), in both 3-AI and I-3Ald is due to the intramolecular charge – transfer transition in the opposite direction. On the other hand, the bands at short wavelengths, at 208, 208 nm ( $^1B_a$ ) and 242, 244 nm ( $^1B_b$ ), most probably result from transitions in the indolic ring.

The spectra of 3-AI (400–190 nm region), recorded in different concentrations of perchloric acid, are shown in Figure 4.



Scheme 2

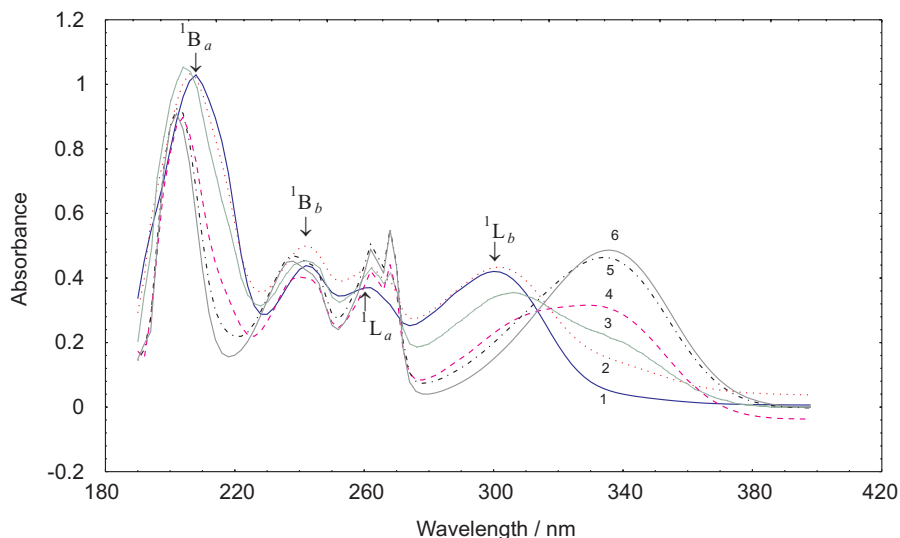


Figure 4. UV absorption spectra of 3-acetylindole ( $4.010^{-5} \text{ mol dm}^{-3}$ ) as a function of perchloric acid concentration. Solvent (bottom to top, at  $\lambda = 340 \text{ nm}$ ) perchloric acid: (1)–1.0; (2)–2.0; (3)–3.0; (4)–4.0; (5)–5.0 and (6)–6.0  $\text{mol dm}^{-3}$ .

Table V presents absorbance *vs.* concentration data for 3-AI in perchloric acid solutions, measured at 290, 300, 320 and 340 nm. A bathochromic shift is observed in the  ${}^1L_b$  bands upon increasing the solvent polarity. The

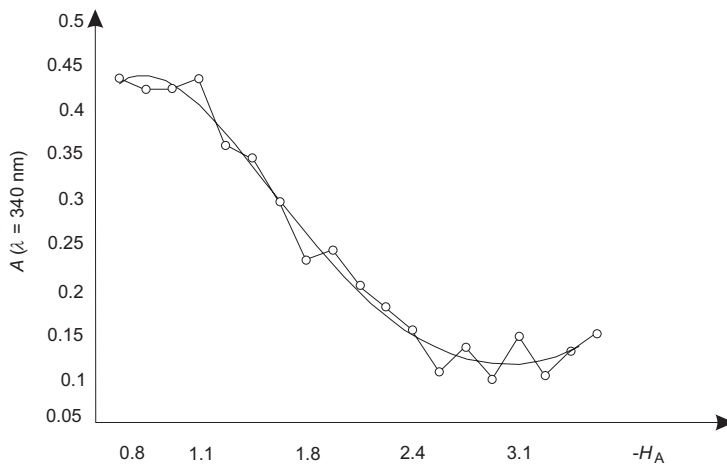


Figure 5. Effect of the  $H_A$  acidity function on the absorbance at 300 nm for 3-acetylindole in perchloric acid.

$^1L_b$  band of 3-AI in perchloric acid of a concentration of  $7.0 \text{ mol dm}^{-3}$  disappears (Table V).

TABLE V

Absorbance (UV spectrum) *vs.* concentration data for 3-acetylindole

$c(\text{HClO}_4)$ $\text{mol dm}^{-3}$	A (290 nm)	A (300 nm)	A (320 nm)	A (340 nm)
1.0	0.2829	0.4204	0.1910	0.0403
1.5	0.2671	0.4212	0.2077	0.0550
2.0	0.2936	0.4326	0.2703	0.1363
2.5	0.2137	0.3587	0.2500	0.1370
3.0	0.2004	0.3422	0.2912	0.2004
3.5	0.1470	0.2944	0.3209	0.2697
4.0	0.0901	0.2289	0.3086	0.2867
4.5	0.1205	0.2399	0.3801	0.3999
5.0	0.0759	0.1992	0.3897	0.4451
5.5	0.1556	0.1727	0.4912	0.5770
6.0	0.0405	0.1495	0.3752	0.4750
6.5	0.0039	0.1021	0.3494	0.4773
7.0	0.0231	0.1304	0.3871	0.5316
7.5	0.0004	0.0940	0.3464	0.5095
8.0	0.0435	0.1416	0.3902	0.5402
8.5	0.0029	0.0972	0.3459	0.5172
9.0	0.0352	0.1246	0.3738	0.5569
9.5	0.0700	0.1441	0.3641	0.5323

The appearance of new bands at 202 ( $^1B_a$ ), 236 ( $^1B_b$ ), 262 ( $^1L_a$ ), 268 and 340 ( $^1L_b$ ) nm may also be noted. The band at 340 nm is probably due to the intramolecular charge-transfer transition from the indolic ring to the protonated carbonyl group.

On the basis of the above results, protonation seems to lower more effectively the energy of the ground state (HOMO) than that of the excited state (LUMO). In the concentration region between  $2.0 \text{ mol dm}^{-3}$  and  $9.5 \text{ mol dm}^{-3}$ , a doublet of bands appears at 262 and 268 nm. This splitting may be associated with one of the out-of-plane C-H bending modes in the indolic ring.

Again, the absorbance plot of 3-AI (measured at  $\lambda = 300 \text{ nm}$ ) *versus*  $H_A$  ( $\text{HClO}_4$ ) shown in Figure 5 gives a clearer picture of the changes that happen in the reaction solution at different  $H_A$  values. The appearance of a plateau in the  $H_A$  range from  $-2.3$  to  $-3.6$  at 300 nm might be explained by the presence of the 3-AI cation.

*UV Spectra of Indole-2-Carboxylic Acid (I-2CA) in Water  
and Perchloric Acid Solutions*

The bands at 202 ( $^1B_a$ ), 218 ( $^1B_b$ ) and 292 nm (overlapped  $^1L_a$  and  $^1L_b$  transitions) are present in the UV-spectrum of I-2CA at 300 K in aqueous solution (Table III). This result is in good agreement with the data by Song and Kurtin.<sup>31</sup> The electronic absorption spectrum of I-2CA 'has lost' the long-wavelength shoulder ( $^1L_b$ -band) present in the spectrum of indole. Thus, the maximum absorption is at 292 nm and the  $^1L_b$  band is now very strongly overlapped with the  $^1L_a$  band. Some evidence for overlapping of the  $^1L_a$  and  $^1L_b$  bands was provided studying the polarized phosphorescence excitation spectrum of I-2CA in an EPA mixture (diethyl ether, isopentane and ethanol; 5:5:2) at 77 K.<sup>31</sup>

The spectrum of this compound, I-2CA, in perchloric acid media (in a wide range from 1.0 to 10.0 mol dm<sup>-3</sup>) does not exhibit very pronounced changes. Minor changes are observed in the position and intensity of the  $^1B_a$ ,  $^1B_b$  and  $^1L_a$  bands. By increasing the concentration of the perchloric acid, the intensity of the  $^1B_a$ ,  $^1B_b$  and  $^1L_a$  bands decreases, showing red shifts at the same time. This suggests the presence of a single absorbing species in the entire range of concentrations. Further studies on the protonation of I-2CA in sulfuric acid are in progress.

*Determination of  $pK_a$  Values Employing the Hammett Acidity  
Function Method*

Using the values for  $H_0$  and  $H_i$  (the function of indoles in perchloric acid with a concentration up to 6.0 mol dm<sup>-3</sup> HClO<sub>4</sub>,<sup>2</sup> the least squares treatment for 15 data pairs gives the following linear equation:

$$H_i = 1.419 H_0 - 0.519 \quad (r = 0.999) . \quad (3)$$

The values for  $H_i$  were obtained by extrapolation of the calculation results for a concentration range of 6.0–11.0 mol dm<sup>-3</sup> perchloric acid. Using the indolic acidity function for perchloric acid  $H_i$ , the dissociation constants ( $pK_a$ ) of 3-MI and D-Trp were calculated graphically and numerically by Eq. (4), analogous to Eq. (1)

$$pK_a = H_i + \log I . \quad (4)$$

In order to determine the ionization ratio  $I = c(BH^+)/c(B)$  in Hammett's equation (4), the absorbances were measured at four analytical wavelengths (Table II). Concentrations of the protonated and unprotonated forms were obtained by solving the system of linear equations:

$$\begin{aligned}
A(\lambda_1) &= \varepsilon(\lambda_1) c(\text{B}) + \varepsilon(\lambda_1) c(\text{BH}^+) \\
A(\lambda_2) &= \varepsilon(\lambda_2) c(\text{B}) + \varepsilon(\lambda_2) c(\text{BH}^+) \\
A(\lambda_3) &= \varepsilon(\lambda_3) c(\text{B}) + \varepsilon(\lambda_3) c(\text{BH}^+) \\
A(\lambda_4) &= \varepsilon(\lambda_4) c(\text{B}) + \varepsilon(\lambda_4) c(\text{BH}^+) .
\end{aligned}
\tag{5}$$

The values  $[d(\log I) / dH_i]$  (denoted as  $m$ ) for 3-MI and D-Trp were similar to those presented in our previous paper and they are close to unity 1 (Table VI). On the other hand,  $d(\log I) / dH_i$  values obtained likewise for 3-AI and I-2CA are very different from those obtained for indole, 3-MI and D-Trp in the same solvent. However, the experimental value of  $d(\log I) / dH_A = 1.02$  for 3-AI is in very good agreement with the theoretical value of 1.00. In fact, the obtained result for 3-AI shows that the position of protonation in 3-AI is at the oxygen atom of the carbonyl group. The results compiled in Table VI indicate that D-Trp is less basic than 3-MI. In addition, the  $pK_a$  values listed in Table VI indicate that monoprotection of 3-MI and D-Trp is complete in 8.0 and 10.0 mol dm<sup>-3</sup> perchloric acid, respectively.

TABLE VI

$pK_a$  values (determined numerically and graphically) for all studied indoles

ions	numerical (HAFM)	graphical (EAM)	graphical (HAFM)	$m$	$m^*$
3-MIH <sup>+</sup>	$-4.0 \pm 0.1$	$-2.8 \pm 0.2$	$-3.9; -4.5^1$	$-0.80$ ( $-0.999$ ) <sup>2</sup>	$1.3 \pm 0.1$ ( $0.999$ ) <sup>2</sup>
D-TrpH <sup>+</sup>	$-6.5 \pm 0.1$	$-4.2 \pm 0.3$	$-5.4$	$-0.84$ ( $-0.981$ )	$0.85 \pm 0.2$ ( $0.983$ )
3-AIH <sup>+</sup>	$-2.8 \pm 0.4$	$-1.2 \pm 0.4$	$-2.8; -1.8^3$	$-1.02^3$ ( $-0.997$ )	$0.6 \pm 0.1$ ( $0.999$ )
I-2CAH <sup>+</sup>	$-5.8 \pm 0.6$	$-2.1 \pm 0.3$	$-7.6; -2.3^3$	$-0.62^3$ ( $-0.987$ )	$0.2 \pm 0.1$ ( $0.967$ )

<sup>1</sup> Ref. 13.

<sup>2</sup> The number in parentheses is the correlation coefficient.

<sup>3</sup> Obtained by the least squares treatment of  $\log I$  vs.  $H_A$ .

As mentioned above, the UV spectral changes of I-2CA, upon increase of the perchloric acid concentration up to 11.0 mol dm<sup>-3</sup>, do not indicate that a significant degree of protonation occurs. Under such conditions, it is impossible to determine  $pK_a$  and  $d(\log I) / dH_A$  for I-2CA with high accuracy. For this reason, we extended our studies on protonation to systems containing other strong acids (e.g. sulfuric acid; the results will be published in a subsequent paper).

*Determination of the  $pK_a$  values by the Excess Acidity Method*

Nowadays, it is well established that the acid – base behavior of weak bases in non-ideal aqueous media may be described by at least two parameters ( $pK_a$  and  $m^*$ ), which must reflect the internal and external stabilization of the acid-base conjugate pairs as a result of the molecular structure itself and the solvation process,<sup>32</sup> respectively. The approach based on the *Excess Acidity Methods* proved to be very useful for this purpose. The dissociation constant ( $pK_a$ ) and solvent parameter ( $m^*$ ) were obtained from Eq. (2) where  $X$  is the Excess Acidity Function.<sup>10</sup>

In this work, the  $X$  values were calculated in the range of 1.0 to 11.0 mol dm<sup>-3</sup> using Eq. (6).

$$X = a_1(z-1) + a_2(z^2-1) + a_3(z^3-1) + a_4(z^4-1) + a_5(z^5-1) \quad (6)$$

where  $z = \text{antilog}(w/80)$  and  $a_1, a_2, a_3, a_4, a_5$  are polynomial coefficients.

The values of perchloric acid fraction (% by weight) were obtained directly from the acid concentration (in mol dm<sup>-3</sup>) using published densities at 298 K.<sup>33</sup>

EAM  $pK_a$  values obtained for the investigated substances (Table VI) in most cases differ from those obtained by HAFM. The  $m^*$  values of 3-MI and D-Trp deviate from the typical values of the carbon base (commonly between 1.7 and 2.2). On the other hand, they are close to the values for nitrogen bases such as anilines (1.0). This behavior of carbon bases 3-MI and D-Trp (C atom being protonated) strongly suggests that the positive charge is almost completely localized on the nitrogen atom. The  $m^*$  value of an oxygen base, such as 3-AI, is in agreement with typical values (in the range of 0.4 – 0.6 units). This behavior of oxygen base 3-AI suggests, once again, that protonation occurs on the carbonyl oxygen atom.

According to the values for  $pK_a$  calculated by the numerical methods, the following order of protonation is inferred:

3-acetylindole > 3-methylindole > D-tryptophan > indole-2-carboxylic acid.

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## SAŽETAK

**Studij protoniranja indol-2-karboksilne kiseline, 3-metilindola, 3-acetilindola i D-triptofana u otopinama perklorne kiseline s pomoću UV spektroskopije***Blagoja S. Andonovski*

Protoniranje indol-2-karboksilne kiseline, 3-metilindola, 3-acetilindola i D-triptofana u otopinama perklorne kiseline proučavano je UV spektroskopijom u području 400–180 nm. Vrijednosti absorpcije mjerene su kod četiri odabrane valne duljine i izračunani su molarni absorpcijski koeficijenti. Iz tih su podataka, primjenom Hammettove jednadžbe, određene  $pK_a$ -vrijednosti. Konstante disocijacije i parametar otapala  $m^*$  dobiveni su primjenom metode povišene kiselosti (Excess Acidity Method). Razmatran je položaj dodatnih protona u protoniranim spojevima.